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Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

99106656.4

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

I.L.C. HATTEN-HECKMAN

DEN HAAG, DEN
THE HAGUE, 10/04/00
LA HAYE, LE

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.:
Application no.:
Demande n°: 99106656.4

Anmeldetag:
Date of filing:
Date de dépôt: 01/04/99

Anmelder:
Applicant(s):
Demandeur(s):
Stymne, Sten, Dr.
26831 Svalöv
SWEDEN

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
Recombinant DNA molecules encoding enzymes of the biosynthetic pathway for the production of triacylglycerol

In Anspruch genommene Prioriät(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

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Am Anmeldetag benannte Vertragstaaten:
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Etats contractants désignés lors du depôt:

Bemerkungen:
Remarks:
Remarques:
The original title of the application reads as follows : Recombinant DNA molecules encoding a new class of enzymes in the biosynthetic pathway for the production of triacylglycerol

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RECOMBINANT DNA MOLECULES ENCODING A NEW CLASS OF ENZYMES IN THE BIOSYNTHETIC PATHWAY FOR THE PRODUCTION OF TRIACYLGLYCEROL

The present invention relates to the isolation, identification and characterization of recombinant DNA molecules encoding enzymes catalysing the transfer of fatty acids from phospholipids to diacylglycerol in the biosynthetic pathway for the production of triacylglycerol.

The invention further relates to novel type of enzymes and their encoding genes for transformation. More specifically, the invention relates to use of a type of genes encoding a not previously described type of enzymes hereinafter designated phospholipid:diacylglycerol acyltransferases (PDAT). This type of genes expressed alone in transgenic organisms will enhance the total amount of oil (triacylglycerols) produced in the cells. The PDAT genes, in combination with a gene for the synthesis of an uncommon fatty acid will, when expressed in transgenic organisms, enhance the levels of the uncommon fatty acids in the triacylglycerols.

There is considerable interest world-wide in producing chemical feedstock, such as fatty acids, for industrial use from renewable plant resources rather than non-renewable petrochemicals. This concept has broad appeal to manufacturers and consumers on the basis of resource conservation and provides significant opportunity to develop new industrial crops for agriculture.

There is a diverse array of unusual fatty acids in oils from wild plant species and these have been well characterised (see e.g. Badami & Patil, 1981). Many of these acids have industrial potential and this has led to interest in domesticating relevant plant species to enable agricultural production of particular fatty acids.

Development in genetic engineering technologies combined with greater understanding of the biosynthesis of unusual fatty acids now makes it possible to transfer genes coding for key enzymes involved in the synthesis of a particular fatty acid from a wild species into domesticated oilseed crops. In this way individual fatty acids can be produced in high purity and quantities at moderate costs.

In all crops like rape, sunflower, oilpalm etc., the oil (i.e. triacylglycerols) is the most valuable product of the seeds or fruits and other compounds like starch, protein, and fibre is regarded as by-products with less value. Enhancing the quantity of oil per weight basis at the expense of other compounds in oil crops would therefore increase the value of crop. If genes, regulating the allocation of reduced carbon into the production of oil can be up-regulated, the cells will accumulate more oil on the expense af other products. Such genes might not only be used in already high oil producing cells such as oil crops but could also induce significant oil production in moderate or low oil containing crops such as e.g. soy, oat, maize, potato, sugarbeats, and turnips as well as in micro-organisms.

Summary of the invention

Many of the unusual fatty acids of interest, e.g. medium chain fatty acids, hydroxy fatty acids, epoxy fatty acids and acetylenic fatty acids, have physical properties that are distinctly different from the common plant fatty acids. The present inventors have found that, in plant species naturally accumulating these uncomman fatty acids in their seed oil (i.e. triacylglycerol), these acids are absent, or present in very low amounts in the membrane (phospho)lipids of the seed. The low concentration of these acids in the membrane lipids is most likely a prerequisite for proper membrane function and thereby for proper cell functions. One aspect of the invention is that seeds of transgenic crops can be made to accumulate high amounts of uncommon fatty acids if these fatty acids are efficiently removed from the membrane lipids and channelled into seed triacylglycerols.

The inventors have identified a novel class of enzymes in plants catalysing the transfer of fatty acids from phospholipids to diacylglycerol in the production of triacylglycerol and, presumably, lysophospholipids and that these enzymes (phospholipid:diacylglycerol acyltransferases abbreviated as PDAT) are involved in the removal of hydroxylated, epoxygenated fatty acids, and probably also other uncommon fatty acids such as medium chain fatty acids, from phospholipids in plants. Further, the same enzyme reaction was shown to be present in microsomal preparations from baker's yeast (*Saccharomyces cerevisiae*). A so called ‚knock out' yeast mutant, disrupted in the respective gene was obtained and microsomal membranes from the mutant was shown to totally lack PDAT activity. Thus, it was proved that the disrupted gene encodes for a PDAT enzyme. In addition, two further genes from *Arabidopsis thaliana* were found

with an amino acid sequence having 42 % identity over 96 amino acids and an amino acid sequence having 47 % identity over 73 amino acids with the yeast enzyme.

In a first embodiment, this invention is directed to nucleic acid sequences that encode a PDAT. This includes sequences that encode biologically active PDATs as well as sequences that are to be used as probes, vectors for transformation or cloning intermediates. The PDAT encoding sequence may encode a complete or partial sequence depending upon the intended use. All or a portion of the genomic sequence, cDNA sequence, precursor PDAT or mature PDAT is intended.

In a different aspect, this invention relates to a method for producing a PDAT in a host cell or progeny thereof, including genetically engineered oil seeds, yeast and moulds or any other oil accumulating organism, via the expression of a construct in the cell. Cells containing a PDAT as a result of the production of the PDAT encoding sequence are also contemplated within the scope of the invention.

In a different embodiment, this invention also relates to methods of using a DNA sequence encoding a PDAT for increasing the oil-content within a cell.

Another aspect of the invention relates to the accommodation of high amounts of uncomman fatty acids in the triacylglycerol produced within a cell, by introducing a DNA sequence producing a PDAT that specifically removes these fatty acids from the membrane lipids of the cell and channel them into triacylglycerol. Plant cells having such a modification are also contemplated herein.

A PDAT of this invention includes any sequence of amino acids, such as a protein, polypeptide or peptide fragment obtainable from a microorganism, animal or plant source that demonstrates the ability to catalyse the production of triacylglycerol from a phospholipid and diacylglycerol under enzyme reactive conditions. By „enzyme reactive conditions“ is meant that any necessary conditions are available in an environment (e.g., such factors as temperature, pH, lack of inhibiting substances) which will permit the enzyme to function.

Other PDATs are obtainable from the specific sequences provided herein. Furthermore, it will be apparent that one can obtain natural and synthetic PDATs, including modified amino acid sequences and starting materials for synthetic-protein modelling from the

exemplified PDATs and from PDATs which are obtained through the use of such exemplified sequences. Modifed amino acid sequences include sequences that have been mutated, truncated, increased and the like, whether such sequences were partially or wholly synthesised. Sequences that are actually purified from plant preparations or are identical or encode identical proteins thereto, regardless of the method used to obtain the protein or sequence, are equally considered naturally derived.

Further, the nucleic acid probes (DNA and RNA) of the present invention can be used to screen and recover „homologous“ or „related“ PDATs from a variety of plant and microbial sources.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1.

PDAT activity in microsomal fractions of S. cerevisiae. Aliquots of lyophilised microsomal membranes (10 nmol phosphatidylcholine) from a wild type yeast (strain YN979) (lane 1-3, 3), a yeast mutant (strain B10280), disrupted in the YNROO8w gene (lane 4-6, 9) or the yeast mutant complemented with a single copy plasmid containing the PDAT gene (lane 7) were assayed for PDAT activity. 2 nmol sn-1-oleoyl-sn-2-[^{14}C]-ricinoleoylphosphatidylcholine (lane 1-7) or sn-1-oleoyl-sn-2-[^{14}C]-oleoyl-phosphatidylcholine (lane 8-9) and 5 nmol of dioleoyl-diacylglycerol (lane 2,5, 7-9) or rac-oleoyl-vernoloyldiacylglycerol (lane 3, 6) were added in benzene solution. The benzene was evaporated under N_2 (g) and 0.1 ml of 50 mM potassium phosphate, pH 7.2, was added. The suspension was thoroughly mixed and after 90 min at 30 °C the lipids were extracted in chloroform and separated an thin layer chromatography on silica gel 60 plates in hexan/dietyletber/acetic acid (35:70:1.5). The radioactive lipids were visualised and quantified an the plates by electronic autoradiagraphy (Instant imager, Packard, US). Abbreviations used: triacylglycerol, TAG, FA, fatty acid (i.e. oleic acid); 1-OH-TAG, monoricinoleoyl-triacylglycerol; 1-OH-1-epTAG, monoricinoleoyl-monovernoleoyl-triacylglycerol and OH-FA, ricinoleic acid.

Brief Description of the SEQ ID:

SEQ ID NO. 1: The amino acid sequence of the yeast ORF YNROO8w from *Saccharomyces cerevisiae*

SEQ ID NO. 2: Amino acid sequence of the region of the *Arabidopsis thaliana* genomic sequence (AC004557).

SEQ ID NO. 3:. Amino acid sequence of the region of the *Arabidopsis thaliana* genomic sequence (AB006704).

SEQ ID NO. 4: The corresponding genomic DNA sequence of the amino acid sequence (SEQ ID NO. 5) of the yeast ORF YNROO8w from *Saccharomyces cerevisiae* .

The present invention can be essentially characterized by the following aspects:

1. Use of a PDAT gene (genomic clone or cDNA) for transformation.
2. Use of a DNA molecule according to item 1 wherein said DNA is used for transformation of any organism in order to be expressed in this organism and result in an active recombinant PDAT enzyme in order to increase oil content of the organism.
3. Use of a DNA molecule of item 1 wherein said DNA is used for transformation of any organism in order to prevent the accumulation of undesirable fatty acids in the membrane lipids.
4. Use according to item 1, wherein said PDAT gene is used for transforming transgenic oil accumulating organisms engineered to produce any uncommon fatty acid which is harmful if present in high amounts in membrane lipids, such as medium chain fatty acids, hydroxylated fatty acids, epoxygenated fatty acids and acetylenic fatty acids.
5. Use according to item 1, wherein said PDAT gene is used for transforming organisms, and wherein said organisms are crossed with other oil accummulating organisms engineered to produce any uncommon fatty acid which is harmful if present in high amounts in membrane lipids, comprising medium chain fatty acids, hydroxylated fatty acids, epoxygenated fatty acids and acetylenic fatty acids.
6. Use according to item 1, wherein the enzyme encoded by said PDAT gene or cDNA is coding for a PDAT with distinct acyl specificity.
7. Use according to item 1 wherein said PDAT encoding gene or cDNA, is derived from *Saccharomyces cereviseae,* or contain nucleotide sequences coding for an amino acid sequence 30% or more identical to the amino acid sequence of PDAT as presented in SEQ. ID. NO. 1.
8. Use according to item 1 wherein said PDAT encoding gene or cDNA is derived from *Saccharomyces cereviseae,* or contain nucleotide sequences coding for an

amino acid sequence 40% or more identicai to the amino acid sequence of PDAT as presented in SEQ. ID. NO. 1.

9. Use according to item 1 wherein said PDAT encoding gene or cDNA is derived from *Saccharornyces cereviseae*, or contain nucleotide sequences coding for an amino acid sequence 60% or more identicai to the amino acid sequence of PDAT as presented in SEQ. ID. NO. 1

10. Use according to item 1 wherein said PDAT encoding gene or cDNA is derived from *Saccharornyces cereviseae*, or contain nucleotide sequences coding for an amino acid sequence 80% or more identical to the amino acid sequence of PDAT as presented in SEQ. ID. NO. 1.

11. Use according to claim 1 wherein said PDAT encoding gene or cDNA is derived from from plants or contain nueleotide sequences coding for an amino acid sequence 40% or more identical to the amino acid sequence of PDAT from *Arabidopsis thaliana* as presented in SEQ. ID. NO. 2 or 3.

12. Transgenic oil accumulating organisms comprising, in their genome, a PDAT gene transferred by recombinant DNA technology or somatic hybridization.

13. Transgenic oil accumulating organisms according to item 12 comprising, in their genome, a PDAT gene having specificity for substrates with particular uncommon fatty acid and the gene for said uneommon fatty acid.

14. Transgenic organisms according to item 12 or 13 whcih are selected from the group consisting of fungi, plants and animals.

15. Transgenic organisms according to item 12 or 13 which are selected from the group of agricultural plants.

16. Transgenic organisms according to item 12 or 13 which are selected from the group of agricultural plants and where said PDAT gene is expressed under the control of a storage organ specific promotor.

17. Transgenic organisms according to item 12 or 13 which are selected from the group of agricultural plants and where said PDAT gene is expressed under the control of a seed promotor.

18. Oils from organisms according to item 12 – 17.

19. A method for altering acyl specificity of a PDAT by alteration of the nucleotide sequence of a naturally occurring encoding gene and as a consequence of this altemation creating a gene encoding for an enzyme witb novel acyl specifity.

20. A protein encoded by a DNA molecule according to item 1 or a functional fragment thereof.

21. A protein of item 20 designated phospholipid:diacylglyceriol acyltransferase.

22. A protein of item 21 which has a distinct acyl specificity.
23. A protein of item 13 having the amino acid sequence as set forth in Fig. 1, 2 or 3 or an amino acid sequence with at least 30 % homology to said amino acid sequence.
24. A protein of item 23 isolated from Saccharomyces cereviseae.

EXAMPLE 1

Determination of substrate utilisation by the acyl-CoA independent acyltransferase in the synthesis of triacylglycerols in microsomal preparations of developing castor bean endosperm and bakers yeast.

Microsomal membranes prepared from developing endosperm of castor bean (Ricinus communis) catalyse the selective transfer of ricinoleoyl-(12-hydroxy-9-octadecenoyl) and vernoloyl(12-epoxy-9-octadecenoyl) groups from both diacylglycerols and phosphatidylcholine into triacylglycerols The substrate utilisation was investigated in castor bean microsomes by using radioactive sn-1-oleoyl-sn-2-[^{14}C]ricinoleoyl-diacylglycerol (sn-2-[^{14}C]-ricinoleoyl-diacylglycerol) or sn-1-oleoyl-sn-2-(^{14}C]-ricinoleoyl-phosphatidylcholine (sn-2-(^{14}C]-ricinoleoyl-phosphatidylchaline) together with different non-radioactive diacylglycerol.

The preparatian of microsomal fractions of developing castor bean endosperm and freeze drying of the microsomes were performed in kown manner. Assays with addition of diacylglycerol and phosphatidylcholine substrates were performed. The results showed that if radioactive sn-2-[^{14}C]ricinoleoyl-diacylglyoerol was used as the only added substrate, 2.8 % of the radio-labelled ricinoleoyl chains were found in triacylglycerol with one ricinoleoyl group, 12.4 % of the radioactivity was found in triacylglycerol-species with two ricinoleoyl groups and only trace amounts were associated with triacylglycerol consisting of three ricinoleoyl groups. If incubations with sn-2-[^{14}C] ricinoleoyl-diacylglycerol were performed in a 1:4 (mol:mol) mixture with non-radioactive diacylglycerol species containing one vernoloyl group, the distribution of radioactivity between different molecular species of triacylglycerol changed only marginally compared to incubations with just radioactive substrate. Only 1.3% of the added ^{14}C-labelled ricinoleoyl groups were metabolised into triacylglycerol species with one ricinoleoyl and one vernoloyl group. Similarly, only marginal changes in the radioactive triacylglycerol molecular species was seen in incubations where *sn*-2-[^{14}C]-

ricinoleoyl-diacylglycerol was mixed with non-labelled divernoloyl-diacylglycerol. However, by adding unlabelled diricinoleoyl-phosphatidylcholine together with sn-2-[^{14}C]-ricinoleoyl-diacylglycerol the radioactivity metabolised into the different triacylglycerol species were substantially altered.

Only trace amounts of radioactivity were detected in triacylglycerol species with one ricinoleoyl chain whereas the radioactivity in triacylglycerol with two ricinoleoyl groups were doubled as compared to incubations with only sn-2-[^{14}C]-ricinoleoyl-diacylglycerol added.

EXAMPLE 2

Transformation and expression of YNROORw gene in yeast

The yeast mutant (strain B 10280) disrupted in the YNROO8w gene, was transformed with the single copy plasmid pFL39 having the PDAT-gene (YNROO8w) under the control of the endogenous promotor region (583 bp 5' untranslated) inserted into the cloning cassette. The transformed yeast was pre-cultivated at 28 ^{0}C for 20 h in defined YNB medium without tryptophane added. Cells were harvested and re-suspended in minimal medium (Meesters eI al., 1996), supplemented with 16 g/l glycerol to the original volume of the growth culture. The culture was further incubated for 24 h after which cells were harvested by centrifugation. Microsomal fraction of the yeast was prepared as described in Example 1 above and was incubated in the presence of sn-2-[^{14}C]-ricinoleoyl-phosphatidylcholine (Fig 1, lane 7). This experiment clearly shows that the PDAT activity could be restored by the expression of the YNROO8w gene in the mutant yeast strain BI0280 normally lacking the PDAT-activity.

The effect of the over-expression of the PDAT gene on the lipid accumulation was studied by transforming the wild-type yeast (strain SCY62) with a plasmid pJN92 containing the PDAT gene (YNR008w) under the control of a GALl-promotor. The transformed yeast was then cultivated at 28 ^{0}C in defined YBN medium lacking uracil. The expression of the PDAT gene was induced by the addition of 2 % *(v/v)* galactose after 10 hours growth and was further incubated for 18 hours. The yeast cells were harvested and the lipid content of the yeast was analysed by thin layer chromatography and gas liquid chromatography. The total lipid content in the yeast with the over-expressed PDAT was 1.3 fold higher that in the control yeast transformed with an empty

plasmid pJN92. The expression of the PDAT gene bhd no effeet on the growth rate as determined by optical density measurements. The elevated lipid content in the yeast transformed with PDAT as compared to the control yeast can be totally accounted for by an 80 % increase seen in the triacylglycerol content. The levels of the polar lipids and sterol esters were not significantly effected by the over-expression of the PDAT gene. Hence, these results clearly demonstrate the use of the PDAT gene in increasing the oil content in transgenic organisms.

SEQUENCE LISTING

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Lys Arg Asp Gly Asn Gly Arg Lys Arg Trp Arg Asp Ser Arg Arg Leu
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Ile Phe Ile Leu Gly Ala Phe Leu Gly Val Leu Leu Pro Phe Ser Phe
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Val Asn Phe Asp Ser Leu Lys Val Tyr Leu Asp Asp Trp Lys Asp Val
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ctc cca caa ggt ata agt tcg ttt att gat gat att cag gct ggt aac 432
Leu Pro Gln Gly Ile Ser Ser Phe Ile Asp Asp Ile Gln Ala Gly Asn
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Tyr Ser Thr Ser Ser Leu Asp Asp Leu Ser Glu Asn Phe Ala Val Gly
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Gly Asp Asp Glu Cys Asp Ser Ser Ala His Phe Arg Lys Arg Leu Trp
195 200 205

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Gly Ser Phe Tyr Met Leu Arg Thr Met Val Met Asp Lys Val Cys Trp
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Leu Lys His Val Met Leu Asp Pro Glu Thr Gly Leu Asp Pro Pro Asn
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Phe Thr Leu Arg Ala Ala Gln Gly Phe Glu Ser Thr Asp Tyr Phe Ile
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gca ggg tat tgg att tgg aac aaa gtt ttc caa aat ctg gga gta att 816
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ggc tat gaa ccc aat aaa atg acg agt gct gcg tat gat tgg agg ctt 864
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Ser Glu Asp Ala Leu Asn Asn Asn Thr Asp Thr Tyr Gly Asn Phe Ile
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cga ttt gaa agg aat acg agc gat gct ttc aac aaa aat ttg aca atg    1392
Arg Phe Glu Arg Asn Thr Ser Asp Ala Phe Asn Lys Asn Leu Thr Met
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aaa gac gcc att aac atg aca tta tcg ata tca cct gaa tgg ctc caa    1440
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gag tta aga aaa aat gag cta cac cac aag cac tgg tcg aat cca atg    1536
Glu Leu Arg Lys Asn Glu Leu His His Lys His Trp Ser Asn Pro Met
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Glu Val Pro Leu Pro Glu Ala Pro His Met Lys Ile Tyr Cys Ile Tyr
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Gly Val Asn Asn Pro Thr Glu Arg Ala Tyr Val Tyr Lys Glu Glu Asp
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Val Phe Leu Thr Glu Gly Asp Gly Thr Val Pro Leu Val Ala His Ser
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Met Cys His Lys Trp Ala Gln Gly Ala Ser Pro Tyr Asn Pro Ala Gly
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Ile Asn Val Thr Ile Val Glu Met Lys His Gln Pro Asp Arg Phe Asp
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ata cgt ggt gga gca aaa agc gcc gaa cac gta gac atc ctc ggc agc    1872
Ile Arg Gly Gly Ala Lys Ser Ala Glu His Val Asp Ile Leu Gly Ser
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gcg gag ttg aac gat tac atc ttg aaa att gca agc ggt aat ggc gat    1920
Ala Glu Leu Asn Asp Tyr Ile Leu Lys Ile Ala Ser Gly Asn Gly Asp
625                 630                 635                 640

ctc gtc gag cca cgc caa ttg tct aat ttg agc cag tgg gtt tct cag    1968
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Asn His Ile His His Gln Gln Gly Leu Gly His Lys Arg Arg Arg Gly
        35                  40                  45

Ile Ser Gly Ser Ala Lys Arg Asn Glu Arg Gly Lys Asp Phe Asp Arg
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Lys Arg Asp Gly Asn Gly Arg Lys Arg Trp Arg Asp Ser Arg Arg Leu
65                  70                  75                  80

Ile Phe Ile Leu Gly Ala Phe Leu Gly Val Leu Leu Pro Phe Ser Phe
                85                  90                  95

Gly Ala Tyr His Val His Asn Ser Asp Ser Asp Leu Phe Asp Asn Phe
            100                 105                 110

Val Asn Phe Asp Ser Leu Lys Val Tyr Leu Asp Asp Trp Lys Asp Val
        115                 120                 125

Leu Pro Gln Gly Ile Ser Ser Phe Ile Asp Asp Ile Gln Ala Gly Asn
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Tyr Ser Thr Ser Ser Leu Asp Asp Leu Ser Glu Asn Phe Ala Val Gly
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Lys Gln Leu Leu Arg Asp Tyr Asn Ile Glu Ala Lys His Pro Val Val
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Met Val Pro Gly Val Ile Ser Thr Gly Ile Glu Ser Trp Gly Val Ile
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Gly Asp Asp Glu Cys Asp Ser Ser Ala His Phe Arg Lys Arg Leu Trp
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Gly Ser Phe Tyr Met Leu Arg Thr Met Val Met Asp Lys Val Cys Trp
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Leu Lys His Val Met Leu Asp Pro Glu Thr Gly Leu Asp Pro Pro Asn
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Phe Thr Leu Arg Ala Ala Gln Gly Phe Glu Ser Thr Asp Tyr Phe Ile
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Ile Gly His Ser Met Gly Ser Gln Ile Ile Phe Tyr Phe Met Lys Trp
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Lys Asp Ala Ile Asn Met Thr Leu Ser Ile Ser Pro Glu Trp Leu Gln
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Val Phe Leu Thr Glu Gly Asp Gly Thr Val Pro Leu Val Ala His Ser
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Met Cys His Lys Trp Ala Gln Gly Ala Ser Pro Tyr Asn Pro Ala Gly
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Ile Asn Val Thr Ile Val Glu Met Lys His Gln Pro Asp Arg Phe Asp
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Ile Arg Gly Gly Ala Lys Ser Ala Glu His Val Asp Ile Leu Gly Ser
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Ala Glu Leu Asn Asp Tyr Ile Leu Lys Ile Ala Ser Gly Asn Gly Asp
625 630 635 640

Leu Val Glu Pro Arg Gln Leu Ser Asn Leu Ser Gln Trp Val Ser Gln
645 650 655

Met Pro Phe Pro Met
660

Claims

1. A recombinant DNA molecule encoding an enzyme catalysing the transfer of fatty acids from phospholipids to diacylglycerol in the biosynthetic pathway for the production of triacylglycerol, wherein said enzyme comprises a amino sequence set forth in SEQ ID NO. 1, SEQ. ID. NO. 2 or SEQ ID NO. 3 or fragments thereof.

2. The DNA molecule of claim 1 wherein said enzyme is designated as phospholipid:diacylglyceriol acyltransferease.

3. A vector comprising a DNA molecule of claim 1 or 2.

4. A vector of claim 3 further comprising a selectable marker gene.

5. A host cell containing a DNA molecule of claim 1 or 2.

6. The host cell of claim 5 which is a plant cell or yeast cell.

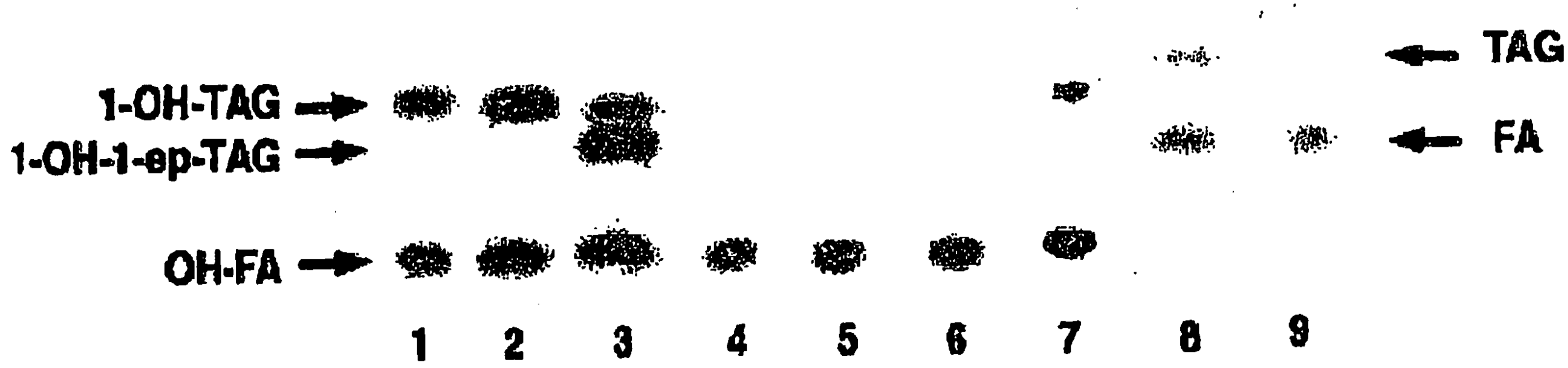
7. A process for the production of transgenic yeast cells, plant cells or plants comprising a) transforming a DNA moleclule of claims 1 or 2 into plant cells or plants; and b) selecting of transformed plant cells or plants having an altered biosynthetic pathway in the production of triacylglycerol.

8. A process of claim 7 wherein the altered biosynthetic pathway is characterised by an increased or altered oil content.

9. A method of claim 7 wherein the altered biosynthetic pathway is characterised by the prevention of accumulation of undesirable fatty acids in the membrane lipids.

10. A protein encoded by a DNA molecule according to claim 1 or 2 or a functional fragment thereof

Fig 1



Abstract of the Disclosure

The present invention relates to the isolation, identification and characterization of recombinant DNA molecules encoding an enzyme catalysing the transfer of fatty acids from phospholipids to diacylglycerol in the biosynthetic pathway for the production of triacylglycerol.